Molecular mechanisms of oxygen sensing
In mammals, O2 sensing occurs at many levels, leading to both acute and chronic adaptation.

Acute seconds.....
The carotid body, which is located at the bifurcation of the internal and external carotid arteries, contains highly specialized chemosensory cells. These glomus cells depolarize in response to reduction in arterial blood PO2 (hypoxemia) resulting in stimulation of the brain stem centers that control the respiratory and cardiovascular systems, which leads to rapid changes in ventilation, heart rate, and blood pressure that serve to increase O2 uptake in the lungs and O2 delivery to the tissues.
2,3 Bisphosphoglycerate (BPG)

- 2,3 BPG is involved in acclimatization to hypoxia as in high altitude
Bisphosphoglycerate (BPG)

- BPG binds in the cavity between β-Hb subunits and Stabilizes T-conformation
2,3 Bisphosphoglycerate (BPG)
Bisphosphoglycerate (BPG)

2,3-BPG is a glycolytic intermediate in RBCs
Most cells contain only a trace of 2,3-BPG, but erythrocytes typically contain 4-5 mM 2,3-BPG.

2,3-bisphosphoglycerate is an important regulator of hemoglobin.

2,3-BPG (for hemoglobin) is made by circumventing the PGK reaction.

2,3-BPG is formed from 1,3-BPG by bisphosphoglycerate mutase.

3-phosphoglycerate is then formed by 2,3-bisphosphoglycerate phosphatase.
Erythrocyte synthesis of 2,3-BPG

Formation and decomposition of 2,3-bisphosphoglycerate in erythrocytes
Erythrocyte synthesis of 2,3-BPG

Hydrolysis of 2,3-BPG by human MIPP1 is sensitive to physiologic alkalosis; This phenomenon provides a homeostatic mechanism for elevating 2,3-BPG levels, thereby enhancing oxygen release to tissues.
Cellular levels of 2,3-BPG in Dictyostelium respond to genetic manipulations of expression.

Levels of 2,3-BPG in wild-type Dictyostelium, in cells in which the mipp1 gene was disrupted -, and in cells in which DdMipp1 was overexpressed +.
The effect of pH on enzyme activity of MIPPI

2,3-BPG as substrate
L’effetto Bohr: pH bassa → bassa affinità → rilascio di $O_2$

- His-H$^+$
- Terminal-NH$_3^+$
The Effect of pH on MIPP1 and Its Regulatory Significance

As hemoglobin releases oxygen, its affinity for H+ increases, causing intracellular alkalinization.

This elevated intracellular pH drives a positive feedback loop, increasing levels of 2,3-BPG, thereby facilitating more oxygen release.
Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.
Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its $\alpha$ Subunit

Eric Huang et al. - JBC 1996

- Quantification mRNA di HIF1
- Quantificazione proteine: HIF1$\alpha$ e HIF1$\beta$
Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its α Subunit

Huang et al. - JBC 1996

Sonda indigerita

HIF1α è espresso a livello di mRNA.

H=hypoxia; N=normoxia

Quantificazione proteine □ Western blot

HIF1α è presente solo in condizioni di ipossia

HIF1β è sempre presente
Struttura di HIF1

Prolina-Idrossilasi

Pro\textsubscript{402} → OH

Pro\textsubscript{564} → OH

Sequenza di idrossilazione
4-hydroxypyrrolidine-2-carboxylic acid

Idrossiprolina
Riconoscimento specifico dell’idrossiprolina da parte del complesso di VHL
The boomerang-shaped CODD peptide (Hif)

CODD: Carboxyl oxygen dependent domain
CODD-contacting residues of VHL (stick models)
Hyp-binding pocket (VHL)

ODD motif

surface of VHL
The hydrogen-bonding network (VHL) involved in binding of the Hyp564 hydroxyl group (Hif)

red sphere = key water molecule
HIF e VHL fanno parte di complessi molecolari con molte componenti
pVHL is the substrate-recognizing component of a multiprotein E3 ubiquitin ligase complex containing elongins C and B, Cullin 2, and the RING-H2 finger protein Rbx-1.
UBIQUITINIZZAZIONE
ES DDR

ES. VHL

ES DDR

Protein intracellular trafficking & recognition

Proteasomal degradation

Signal transduction Protein interaction
UBIQUITININA
Struttura HIF
HIF-2α

HIF-2β (ARNT)

PAS, PAS-B (interaction domains)

bHLH (DNA Binding domain)
bHLH (DNA Binding domain
PAS,PAS-B (interaction domains

major-groove DNA interactions

minor-groove DNA interactions
DNA-bound HIF-α–ARNT structures.
cancer-related mutations in HIF-1α
### Genere: carcinoma endometrio

cancer-related mutations in HIF-2α and HIF-1α

<table>
<thead>
<tr>
<th>Location</th>
<th>Possible Role</th>
<th>Primary Tissue (Subtype)</th>
<th>Associated Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>K18E</td>
<td>bHLH α1</td>
<td>DNA interaction</td>
<td>Stomach</td>
</tr>
<tr>
<td>A23V</td>
<td>bHLH α1</td>
<td>DNA interaction</td>
<td>Endometrium</td>
</tr>
<tr>
<td>V47M</td>
<td>bHLH α1-α2 loop</td>
<td>Interface 6 (bHLH/PAS-A)</td>
<td>Central nervous system (brain)</td>
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<tr>
<td>F98L</td>
<td>PAS-A Aβ</td>
<td>Internal stability</td>
<td>Large intestine (colon)</td>
</tr>
<tr>
<td>R166L</td>
<td>PAS-A Gβ</td>
<td>Internal stability</td>
<td>Kidney</td>
</tr>
<tr>
<td>I223M</td>
<td>PAS-A Λμ</td>
<td>Interface 2 (PAS-A/PAS-A)</td>
<td>Lung</td>
</tr>
<tr>
<td>H248N</td>
<td>PAS-B Aα</td>
<td>Internal stability</td>
<td>Large intestine (colon)</td>
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<tr>
<td>R275H</td>
<td>PAS-B Dα-Eα loop</td>
<td>Internal stability</td>
<td>Cervix</td>
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<tr>
<td>A277P</td>
<td>PAS-B Eα</td>
<td>Internal stability</td>
<td>Lung</td>
</tr>
<tr>
<td>E279V</td>
<td>PAS-B Eα</td>
<td>Internal stability</td>
<td>Liver</td>
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<td>K19Q</td>
<td>bHLH α1</td>
<td>DNA interaction</td>
<td>Endometrium</td>
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<td>R30Q</td>
<td>bHLH α1</td>
<td>DNA interaction</td>
<td>Skin</td>
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<tr>
<td>L54I</td>
<td>bHLH α1-α2 loop</td>
<td>Interface 6 (bHLH/PAS-A)</td>
<td>Kidney</td>
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<tr>
<td>V116E</td>
<td>PAS-A Cα</td>
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<tr>
<td>M120T</td>
<td>PAS-A Cα</td>
<td>Internal stability</td>
<td>Large intestine (colon)</td>
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<tr>
<td>M171I</td>
<td>PAS-A Gβ</td>
<td>Internal stability</td>
<td>Kidney</td>
</tr>
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<td>M250I</td>
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<td>Internal stability</td>
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<tr>
<td>L262S</td>
<td>PAS-B Cα</td>
<td>Internal stability</td>
<td>Skin</td>
</tr>
<tr>
<td>V341I</td>
<td>PAS-B Iβ</td>
<td>Internal stability</td>
<td>Endometrium</td>
</tr>
</tbody>
</table>
Le prolil idrossilasi (PHD) hanno ruolo chiave e sono finemente regolate.
PHDs require 2-oxalglutarate and the cofactors oxygen and iron to hydroxylate substrates, such as hypoxia inducible factor (HIF)-1α. Inhibitory factors of PHD function include the metabolic intermediates succinate and fumarate, or 2-hydroxyglutarate (2-HG), which compete with 2-oxalglutarate (2-OG); divalent metal ions such as Co2+ or Ni2+, which compete with Fe2+ binding to PHDs; and reactive oxygen species (ROS), which can disrupt oxygen interaction with PHDs.

PCBP1 not only delivers iron to ferritin for intracellular iron storage, but also delivers Fe2+ to PHDs, which is necessary for their activation and function.

Fe2+, which binds the proline substrate and the oxygen molecule, undergoes oxidation in the Fenton reaction. Ascorbate/glutathione maintains iron in the active site of PHDs in the reduced (ferrous) state.

Emerging novel functions of the oxygen-sensing prolyl hydroxylase domain enzymes Brian W. Won
Concentrations of oxygen in tissues
- range 10–30 μM-

below the Km for oxygen of the hydroxylases

Concentrations of oxygen is limiting for enzyme activity over the entire physiological range.
HIF  Metabolismo e Mitocondrio
HIFα Control of Cell Metabolism
Figure 1. Central Role of PHD Prolyl Hydroxylases and the HIF Transcription Factor in Response to Hypoxia

Oxygen Sensing by Metazoans: The Central Role of the HIF Hydroxylase Pathway William G. Kaelin Jr., Peter J. Ratcliffe
null, Volume 30, Issue 4, 2008, 393–402
(B) **Under low oxygen tension** HIFa associates with HIFb. The heterodimer binds to a core consensus sequence at the promoters of HIF-responsive genes, and upon binding to the coactivators p300/CBP and PKM2, initiates transcription. The interaction between HIFa and p300 may be regulated by a variety of factors that to influence the transcriptional activity.

(PHD, prolyl-hydroxylase domain-containing enzyme; SIRT, sirtuin; FIH, factor inhibiting HIF; CBP, Creb-binding protein; OH, hydroxyl group; STAT3, signal transducer and activator of transcription 3; ub, ubiquitin moiety; EloB/C, elongins B and C; Cul2, cullin 2; pVHL, von Hippel-Lindau protein; ROS, reactive oxygen species; CITED2/4, CBP/p300 interacting transactivator; PKM2, pyruvate kinase isoform M2; hnRNPs, heterogeneous nuclear ribonucleoproteins).
Mutazioni nella Pathway oxygen sensing
Policitemia di Chuvash
Ang et al. Nature Genetics 2002

- Policitemia autosomica recessiva trovata in Russia

### Table 1 - Biochemical parameters in Chuvash polycythemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Individuals with Chuvash polycythemia (n = 20)</th>
<th>Unaffected relatives (n = 51)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoietin (mIU ml⁻¹)</td>
<td>61.9 ± 12.8</td>
<td>6.4 ± 6.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum ferritin (ng ml⁻¹)</td>
<td>19 (15–24)</td>
<td>28 (25–32)</td>
<td>0.2</td>
</tr>
<tr>
<td>Serum iron (µg dL⁻¹)</td>
<td>64 ± 15</td>
<td>81 ± 9</td>
<td>0.4</td>
</tr>
<tr>
<td>Total iron binding capacity (µg dL⁻¹)</td>
<td>427 ± 18</td>
<td>346 ± 10</td>
<td>0.001</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>16 ± 4</td>
<td>24 ± 2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Sequenziamento gene von Hippel Lindau (VHL) □ C/T transition, Arg/Trp200 (Pazienti omozigoti)
Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia
Sonny O. Ang
Nature genetics 2002, volume 32 no. 4 pp 614 - 621

20% O₂:
- Livelli di proteina VHL normali in mutato e Wt
- Livelli di HIF1α maggiori nei soggetti affetti

Western blot, 5 pazienti + 5 controlli
Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia
Sonny O. Ang
Nature genetics 2002, volume 32 no. 4 pp 614 - 621

La forma ubiquitinizzata è meno presente nelle cellule del paziente

Mutazione Arg200Trp:
- Ridotta ubiquitinizzazione di HIF1α
- Aumentata espressione del gene Epo  → policitemia

V= controllo (Wild type)
H= eterozigote
P1= paziente (omozigote)
Increased survival, proliferation, and differentiation of erythroid progenitor cells

EPOR

EPO

Type 1
EPOR GoF (multiple)

HIF-1α

HIF-2α

O2

PHD2

Type 2
VHL LoF
R200W

Type 4
GoF
G537W, G537R, M535V, M5351

VHL

HIF-α
Pro-OH

Proteasomal degradation

Type 3
LoF
P317R
R371H

HIF-α
Pro-OH
Lys-Ubiₙ